1: FEBS Lett. 2003 Mar 13;538(1-3):173-7.

Related Articles, L

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Identification of mammalian orthologs associates PYPAF5 with distinct functional roles.

Albrecht M, Domingues FS, Schreiber S, Lengauer T.

Max-Planck-Institute for Informatics, Stuhlsatzenhausweg 85, 66123 Saarbrucken, Germany. mario.albrecht@mpi-sb.mpg.de

PYRIN- and CARD-containing proteins belong to a recently identified protei family involved in the regulation of apoptosis and inflammatory processes. Variations in the gene products of the family members PYPAF1 and NOD2/CARD15 have been associated with several autoinflammatory disease We could identify the mouse orthologs of PYPAF1, PYPAF5, NOD1, NOD2 and the rat ortholog of PYPAF5. Intriguingly, we found that PYPAF5 has be reported previously not only as regulator of NF-kappaB and caspase-1, but al as angiotensin II and vasopressin receptor. In particular, based on a comprehensive sequence analysis, we propose a structural model for this hormone receptor that is different from the model suggested previously.

PMID: 12633874 [PubMed - indexed for MEDLINE]

Display Abstract Show: 20 Sort Send to Text

FEBS 27047

FEBS Letters 538 (2003) 173–177

Identification of mammalian orthologs associates PYPAF5 with distinct functional roles

Mario Albrecht[a,*], Francisco S. Domingues[a], Stefan Schreiber[b], Thomas Lengauer[a]

[a] *Max-Planck-Institute for Informatics, Stuhlsatzenhausweg 85, 66123 Saarbrücken, Germany*
[b] *First Department of Medicine, Christian-Albrechts-Universität Kiel, Schittenhelmstr. 12, 24105 Kiel, Germany*

Received 18 January 2003; revised 4 February 2003; accepted 7 February 2003

First published online 21 February 2003

Edited by Gunnar von Heijne

Abstract PYRIN- and CARD-containing proteins belong to a recently identified protein family involved in the regulation of apoptosis and inflammatory processes. Variations in the gene products of the family members PYPAF1 and NOD2/CARD15 have been associated with several autoinflammatory diseases. We could identify the mouse orthologs of PYPAF1, PYPAF5, NOD1, NOD2 and the rat ortholog of PYPAF5. Intriguingly, we found that PYPAF5 has been reported previously not only as regulator of NF-κB and caspase-1, but also as angiotensin II and vasopressin receptor. In particular, based on a comprehensive sequence analysis, we propose a structural model for this hormone receptor that is different from the model suggested previously.

Key words: PYRIN-/CARD-containing protein; Mammalian ortholog; Inflammation; Hormone receptor; G-protein coupled receptor; Structure prediction

1. Introduction

The expressed human proteins PYPAF1, PYPAF5, NOD1, and NOD2, all with as yet unknown biological functions, are members of the same protein family putatively involved in the regulation of apoptosis and inflammatory immune responses [1–4]. The family members share a similar domain architecture consisting of one or several N-terminal PYRIN (as in the protein pyrin) or CARD (caspase activating recruitment) homologous domains, one intermediate NTPase oligomerization domain, and one C-terminal LRR (leucine-rich repeat) domain (Fig. 1).

Several missense mutations in the PYPAF1 and NOD2 genes have been identified to be independently associated with clinically distinct diseases, which, however, have shared pathophysiological characteristics of autoinflammatory processes [5]. Genetic variations in the PYPAF1 gene have been linked to Muckle–Wells syndrome, familial cold autoinflammatory syndrome, also known as familial cold urticaria, and chronic infantile neurological cutaneous and articular syndrome. Variants in the NOD2 gene have been found to confer susceptibility to Blau syndrome and one of the two main types of chronic inflammatory bowel disease, Crohn's disease.

Experimental data suggests that the described family members participate in the upstream regulation of pro-inflammatory signaling pathways via the activation of NF-κB and procaspase-1 through the PYRIN/CARD domains; caspase-1 is known to process pro-interleukin-1β and pro-interleukin-18 into active cytokines. The generic function of the LRR domain is still unknown, but the domain is proposed to work as a receptor in analogy to the LRR domain of mammalian transmembrane Toll-like receptors and the NTPase-LRR domains of cytoplasmic plant disease resistance gene products [6–9]. The LRR domain of NOD1 and NOD2 appears to mediate the responsiveness to bacterial lipopolysaccharides [10].

In the following, we will briefly report the computational identification of the mouse orthologs of human PYPAF1, NOD1, NOD2, which also allowed us to discover the mouse and rat ortholog of human PYPAF5. We will then focus on a detailed discussion of two seemingly contradictory functional roles that have been associated previously with human and rat PYPAF5. This protein has been reported to work as solely cytosolic NF-κB activator and as seven-transmembrane hormone receptor. Particularly, we will present structural evidence that the LRR domain of PYPAF5 does not contain any transmembrane helix in contrast to another proposed G-protein coupled receptor (GPCR) model.

2. Materials and methods

Sequence searches in the NCBI NR and SWISS-PROT/TrEMBL databases were performed by the BLAST suite of programs. We also used the genomic synteny view and other tools such as gene prediction programs, which are offered by the Ensembl Project and the UCSC Genome Browser, to determine the mammalian orthologs of human genes and the corresponding protein sequences. In addition, we could find the rat ortholog of human PYPAF5 via the whole genome comparative analysis of the Berkeley Genome Pipeline. Expressed sequence tags and other sequence fragments contained in NCBI databases give sufficient evidence for the expression of the identified genes.

Sequence alignments were computed by CLUSTAL W and improved by minor manual modifications. Figures with sequence alignments were prepared using the web tool ESPript. The performance of seven of the used transmembrane prediction methods (DAS, HMMTOP2, PHDhtm, PRED-TMR2, SOSUI, TMHMM2, TopPred2) has been benchmarked comprehensively [11] and reaches an accuracy of over 80 up to 99%. Generally, at least one of the seven methods is able to recognize several transmembrane helices of some

*Corresponding author.
E-mail addresses: mario.albrecht@mpi-sb.mpg.de (M. Albrecht), doming@mpi-sb.mpg.de (F.S. Domingues), s.schreiber@mucosa.de (S. Schreiber), lengauer@mpi-sb.mpg.de (T. Lengauer).

Abbreviations: LRR, leucine-rich repeat; GPCR, G-protein coupled receptor; AVR, angiotensin II and arginine vasopressin receptor; AII, angiotensin II; AVP, arginine vasopressin; RNI, ribonuclease inhibitor

0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00161-3

GPCR properly. Web links to online bioinformatics services utilized in this study are listed in Web Note A (the online version of this article contains supplementary material).

3. Results and discussion

3.1. Identification of mammalian orthologs

Using gene prediction tools and additional information contained in genome and sequence databases, we could identify the mouse orthologs of PYPAF1, PYPAF5, NOD1, NOD2, and the rat ortholog of PYPAF5. Sequence alignments of the PYPAF1/PYPAF5 and the NOD1/NOD2 orthologs (Web figs. A and B, respectively) reveal a high degree of sequence identity of over 75% between corresponding species orthologs (Web Table A).

3.2. Functional roles of PYPAF5

We discovered that PYPAF5 has been associated in the literature with two distinct functional roles and cellular localizations in different cell types. Like PYPAF1, human PYPAF5 has been reported to be recruited by the PYRIN-CARD protein ASC to distinct cytoplasmic loci through the PYRIN domain. It synergistically activates NF-κB and procaspase-1 when co-expressed with ASC [4]. In consistency with the observed involvement of human PYPAF5 in inflammatory signaling, its expression has been described to be restricted to peripheral blood leukocytes.

We also identified an incomplete sequence fragment of rat PYPAF5, which has been obtained from cDNA, encompassing solely the LRR domain. Surprisingly, it has been reported previously from renal epithelial cells as transmembrane dual angiotensin II (AII) and arginine vasopressin (AVP) receptor (AVR) with characteristics of AT_1 and V_2 isoreceptor subtypes coupled to the adenylate cyclase system [12,13].

3.3. Transmembrane helices

The authors of the original publication on AVR [12], lacking the complete PYPAF5 sequence, proposed a hormone receptor model of a GPCR [14] with seven transmembrane helices for the AVR (the LRR domain of PYPAF5) based on the Kyte–Doolittle hydropathy index. To investigate this issue further, we plotted this index both for the complete rat PYPAF5 protein sequence and only for its LRR domain (Fig. 3A and B, respectively). However, the hydropathy index in the plot for the LRR domain is always under 1.2 and never exceeds the hydrophobicity threshold of 1.6 characteristic of transmembrane helices as advised by Kyte and Doolittle [15]. We obtained similar results for the LRR domain with other hydropathy indices and parameters, using the tool ProtScale.

It seems that the authors on AVR ignored the common use of a hydrophobicity threshold for potential transmembrane regions. Above all, none of 12 state-of-the-art prediction servers (see Web Table A) detects any transmembrane helix in the AVR sequence with a reliable confidence value. In accordance with these findings, we did not detect any homology to other GPCRs and AII or AVP receptors deposited in the GPCR database.

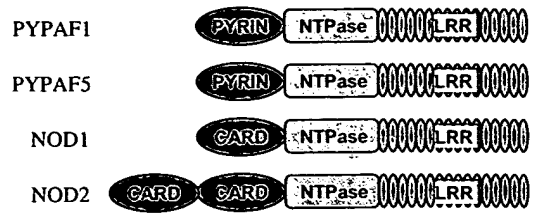


Fig. 1. Domain architectures of PYPAF1, PYPAF5, NOD1, and NOD2.

In contrast, plotting the hydropathy index for the complete PYPAF5 protein sequence indicates a rather hydrophobic region between the NTPase and LRR domain from N376 to M400 (Fig. 3A), possibly containing a single-transmembrane helix. This finding is confirmed for the same region with a reliable score of 2.0 by the more advanced transmembrane prediction method TopPred2. However, the TMHMM2 prediction server returns a less significant probability value of 0.01, and similar results are obtained from other state-of-the-art transmembrane prediction servers. As the identified hydrophobic region is highly conserved in PYPAF1 (Web fig. A), which has been described only as a cytosolic protein like NOD1 and NOD2 [3], we cannot predict any transmembrane helix in PYPAF5 with confidence.

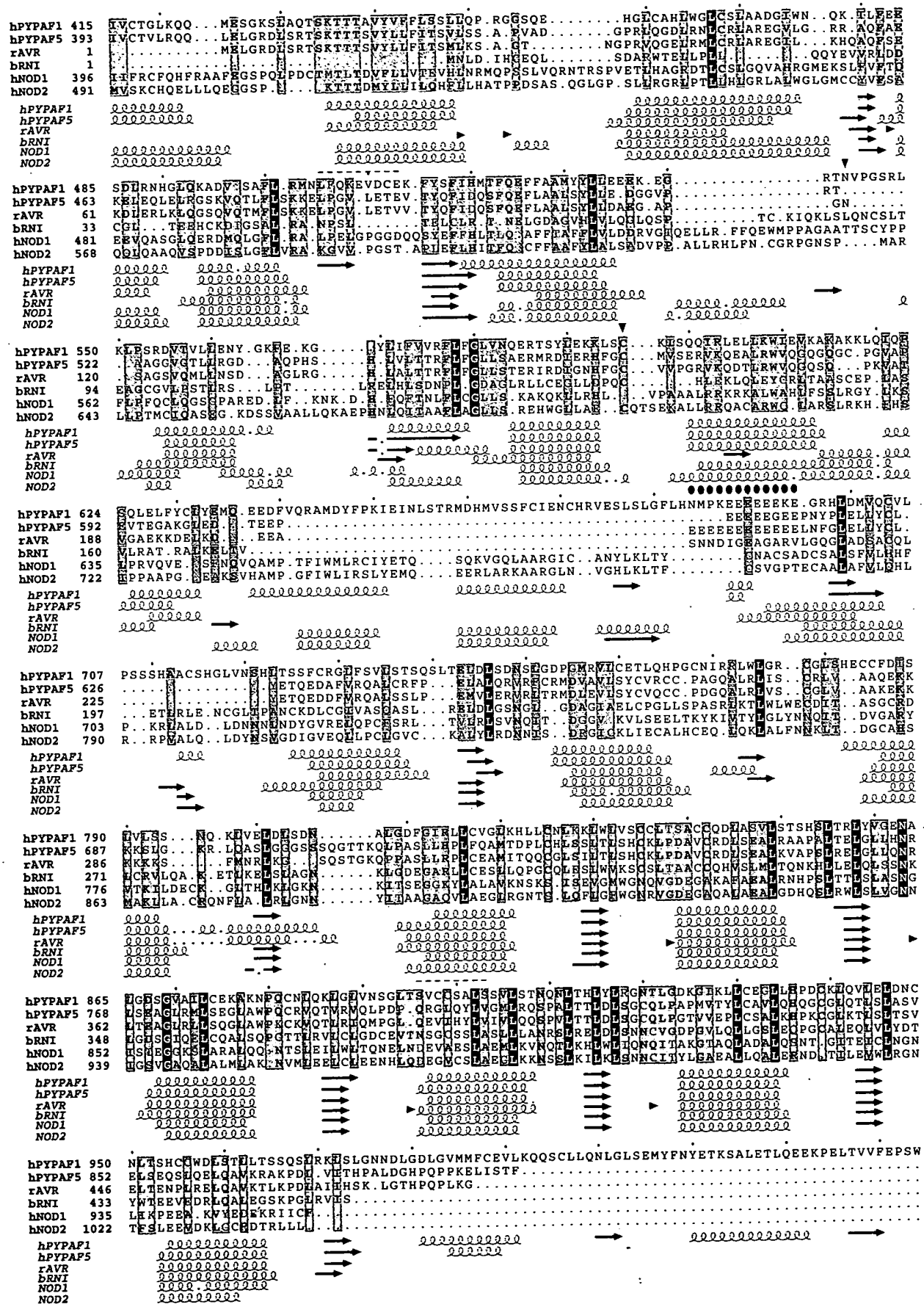
3.4. Multiple sequence alignment

Finally, we assembled a multiple sequence alignment of the LRR domains of human PYPAF1, human PYPAF5, rat AVR/PYPAF5, human NOD1, human NOD2. We annotated the alignment with the secondary structures predicted by the PSIPRED server (Fig. 2). In addition, we included the sequence and the crystallographically determined secondary structure of the bovine ribonuclease inhibitor (RNI) of ribonuclease A into the alignment. A BLAST search with the AVR sequence in the PDB database returns a significant *E*-value of 10^{-20} to RNI. This result implicates an evolutionary relationship and a similar three-dimensional structure. In addition, the secondary structure annotations of the aligned proteins agree well with each other (Fig. 2). A sequence identity matrix derived from the multiple sequence alignment gives significantly high values (Web Table B).

Apparently, the secondary structure prediction of AVR disagrees substantially with the seven-transmembrane model proposed in the original publication, but matches to the RNI structure.

→

Fig. 2. Multiple sequence alignment of the LRR domains of human PYPAF1, human PYPAF5, rat PYPAF5/AVR, bovine RNI, human NOD1, and human NOD2. The secondary structure of RNI and the corresponding predictions by the PSIPRED server for the other proteins are depicted in the lower part (α-helices are represented by curled lines, β-strands by arrows). The alignment columns with strictly conserved residues are highlighted in dark gray boxes, those in which more than 65% of the residues are physico-chemically equivalent are shown in light gray boxes. Dashed lines above the alignment indicate the positions of the putative AVP and AII binding sites near the N- and C-terminus, respectively, of AVR. Solid circles denote a conserved, highly acidic region in PYPAF1 and PYPAF5. Triangles mark the two sequence variations N119S and C163R associated with sodium-induced AVR dysfunction. The sequence and the secondary structure of bovine RNI was obtained from the DSSP database via the PDB identifier 1dfj, chain I. The SWISS-PROT/TrEMBL or accession numbers of the aligned proteins are as follows: human PYPAF1/CIAS1/CRYOPYRIN/NALP3, Q96P20; human PYPAF5, P59044; rat PYPAF5/AVR, MGI: 2448892/Q63035; human NOD1/CARD4, Q9Y239; NOD2/CARD15/IBD1, Q9HC29.



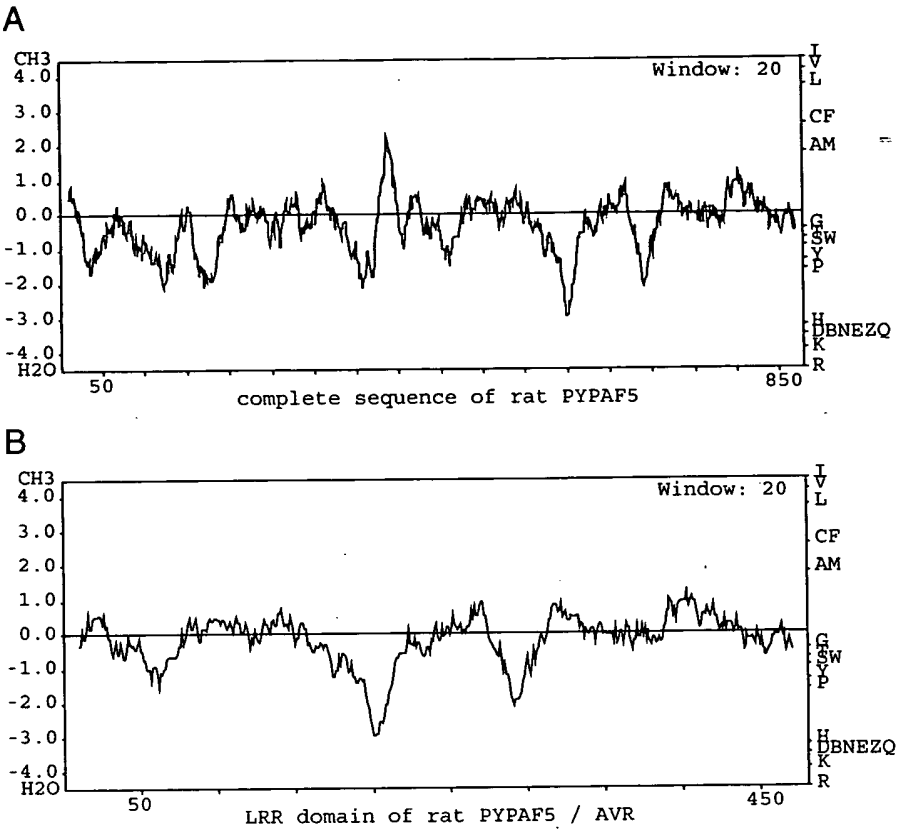


Fig. 3. Plot of the Kyte–Doolittle hydropathy index of rat PYPAF5 (using the averaging-window size of 20 residues as in the original publication on AVR [12]). A: Complete protein sequence of PYPAF5, including a single transmembrane region with positive hydrophobicity values over the common threshold 1.6. B: LRR domain of PYPAF5 (corresponding to the AVR sequence), excluding the single transmembrane region.

The SCOP classification of protein structures indicates that the horseshoe fold of the RNI is also shared by other proteins of distinct functions such as the Ran GTPase activating protein RanGAP [16]. Interestingly, Ran has been observed to be involved in LPS-mediated signal transduction [17], which may provide some functional link between NOD1/NOD2 and RanGAP. Apart from that, the LRR domain of PYPAF1 and PYPAF5 contains the unusual, but conserved sequence feature of a highly acidic region (Fig. 2) as also described in the potato R gene product HERO with broad spectrum resistance to cyst nematodes [18]. This finding strongly supports the evolutionary relationship of the innate immune system of mammals and plants.

Based on sequence similarity to putative binding sites of other AII and AVP receptors and experimental observations on AVR, Ruiz-Opazo et al. suggested two hormone binding sites for AII and AVP in the LRR domain of PYPAF5 [12]. Because the identified regions appear moderately conserved in the multiple sequence alignment of LRR domains (Fig. 2), other family members homologous to PYPAF5 may also recognize AII and AVP. Interestingly, it has been hypothesized that AII and AVP are involved in apoptosis and neuroendocrine immunomodulation, respectively [19,20]. Apart from that, the sequence variations N119S and C163R of AVR, which exhibit sodium-induced receptor dysfunction in a hypertension rat model [21], seem to be preserved in some other homologs of PYPAF5 (Fig. 2).

4. Conclusions

The identified mouse and rat orthologs of human PYRIN- and CARD-containing proteins can be helpful, for instance, in establishing animal models of the involved autoinflammatory diseases. In addition, we propose a new structural model for the renal AVR/PYPAF5 with an extracellular LRR domain, whose three-dimensional fold is similar to the structure of RNI. Our results challenge the previous model of PYPAF5 as a seven-transmembrane glycoprotein receptor [22]. Apart from that, PYPAF5 has been reported as cytoplasmic protein and transmembrane receptor, associated with distinct functions [4,12]. This contradiction should be resolved by additional experimental results on the cellular locations and functional roles of PYPAF5.

Acknowledgements: We are grateful to Gert Vriend for helpful discussions. Part of this research has been funded by the Deutsche Forschungsgemeinschaft (DFG) under contract numbers ZI 616/2 and LE 491/14-1 and by the National Genome Research Network (NGFN).

References

[1] Gumucio, D.L., Diaz, A., Schaner, P., Richards, N., Babcock, C., Schaller, M. and Cesena, T. (2002) Clin. Exp. Rheumatol. 20, S45–S53.
[2] Harton, J.A., Linhoff, M.W., Zhang, J. and Ting, J.P. (2002) J. Immunol. 169, 4088–4093.

[3] Inohara, N., Ogura, Y. and Nuñez, G. (2002) Curr. Opin. Microbiol. 5, 76–80.
[4] Grenier, J.M., Wang, L., Manji, G.A., Huang, W.J., Al-Garawi, A., Kelly, R., Carlson, A., Merriam, S., Lora, J.M., Briskin, M., DiStefano, P.S. and Bertin, J. (2002) FEBS Lett. 530, 73–78.
[5] McDermott, M.F. (2002) Trends Mol. Med. 8, 550–554.
[6] Shirasu, K. and Schulze-Lefert, P. (2000) Plant Mol. Biol. 44, 371–385.
[7] Cohn, J., Sessa, G. and Martin, G.B. (2001) Curr. Opin. Immunol. 13, 55–62.
[8] Nürnberger, T. and Scheel, D. (2001) Trends Plant Sci. 6, 372.
[9] Girardin, S.E., Sansonetti, P.J. and Philpott, D.J. (2002) Trends Microbiol. 10, 193–199.
[10] O'Riordan, M., Yi, C.H., Gonzales, R., Lee, K.D. and Portnoy, D.A. (2002) Proc. Natl. Acad. Sci. USA 99, 13861–13866.
[11] Chen, C.P., Kernytsky, A. and Rost, B. (2002) Protein Sci. 11, 2774–2791.
[12] Ruiz-Opazo, N., Akimoto, K. and Herrera, V.L. (1995) Nat. Med. 1, 1074–1081.
[13] Gonzalez, C.B., Herrera, V.L. and Ruiz-Opazo, N. (1997) Hypertension 29, 957–961.
[14] Pierce, K.L., Premont, R.T. and Lefkowitz, R.J. (2002) Nat. Rev. Mol. Cell Biol. 3, 639–650.
[15] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[16] Kobe, B. and Kajava, A.V. (2001) Curr. Opin. Struct. Biol. 11, 725–732.
[17] Wong, P.M., Kang, A., Chen, H., Yuan, Q., Fan, P., Sultzer, B.M., Kan, Y.W. and Chung, S.W. (1999) Proc. Natl. Acad. Sci. USA 96, 11543–11548.
[18] Ernst, K., Kumar, A., Kriseleit, D., Kloos, D.U., Phillips, M.S. and Ganal, M.W. (2002) Plant J. 31, 127–136.
[19] Lucius, R., Gallinat, S., Busche, S., Rosenstiel, P. and Unger, T. (1999) Cell. Mol. Life Sci. 56, 1008–1019.
[20] Chikanza, I.C. and Grossman, A.S. (1998) Br. J. Rheumatol. 37, 131–136.
[21] Ruiz-Opazo, N., Lopez, L.V. and Herrera, V.L. (2002) Mol. Med. 8, 24–32.
[22] Jiang, X., Dreano, M., Buckler, D.R., Cheng, S., Ythier, A., Wu, H., Hendrickson, W.A. and el Tayar, N. (1995) Structure 3, 1341–1353.